Increases in human plasma antioxidant capacity after consumption of controlled diets high in fruit and vegetables[1–3]

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ABSTRACT

Background: The putative beneficial effects of an increased consumption of fruit and vegetables have been associated with antioxidant nutrients. However, the effect of fruit and vegetable consumption on the overall antioxidant status in humans is unclear.

Objective: The objective of this study was to investigate whether a diet rich in fruit and vegetables would affect the antioxidant capacity of human plasma.

Design: Thirty-six healthy nonsmokers resided in a metabolic research unit and consumed 2 sets of controlled diets. Diet A contained 10 servings of fruit and vegetables each day for 15 d. Diet B was the same as diet A, except diet B also provided 2 servings of broccoli each day on days 6–10. There was a free-living period of a minimum of 6 wk between the 2 experiments using either diet A or diet B. Fasting plasma antioxidant capacity, measured as oxygen radical absorbance capacity (ORAC), and α-tocopherol concentrations were determined on days 1, 6, 11, and 16.

Results: The fasting baseline plasma ORAC of these subjects was significantly correlated with their estimated daily intake of total antioxidants from fruit and vegetables during the previous year. Plasma ORAC of these subjects was significantly increased by both diets A and B. This increase in ORAC could not be explained by the increase in the plasma α-tocopherol concentration.

Conclusion: Increased consumption of fruit and vegetables can increase the plasma antioxidant capacity in humans. *Am J Clin Nutr* 1998;68:1081–7.

KEY WORDS Antioxidant capacity, fruit, vegetables, diet, humans, Trolox, free radicals, α-tocopherols

INTRODUCTION

Increased consumption of fruit and vegetables has been associated with protection against various diseases, including cancers (1–3) and cardio- and cerebrovascular diseases (4, 5). It is not known what dietary constituents are responsible for this association, but it is often assumed that antioxidants contribute to the protection (6–13). However, the effects of increased consumption of fruit and vegetables on the overall antioxidant status is not known, and the results from intervention trials have not been conclusive regarding the protection of supplementation with pure antioxidants (14, 15).

It is therefore plausible that the putative beneficial effects of a high intake of fruit and vegetables on the risk of diseases may not result exclusively from the action of antioxidants, such as the well-characterized vitamins E and C or β-carotene. Rather, they may result from the action of lesser known compounds or from a concerted action of a combination of different antioxidants present in these foods. When 8-hydroxydeoxyguanosine (8OHdG), a product that arises from free radical damage to DNA or to the DNA precursor pool, was measured in human urine, it was found that neither dietary β-carotene supplementation (16) nor supplementation with vitamins E or C or coenzyme Q10 (17) changed the amount of 8OHdG excreted. However, human volunteers fed Brussels sprouts showed a significant decrease in 8OHdG excretion (18). Brussels sprouts are rich in nonvitamin antioxidants, as shown by using the oxygen radical absorbance capacity (ORAC) assay (19), which suggests that the observed decrease in 8OHdG excretion may be associated with other compounds with antioxidant properties.

In this study, we determined whether human plasma antioxidant capacity responded to the ingestion of diets rich in fruit and vegetables in a controlled setting. The antioxidant capacity was measured by using the ORAC assay, which is one of the methods developed recently to assess the total antioxidant activity of a biological sample. The ORAC assay is, to date, the only method that takes a free radical reaction to completion and uses an area-under-the-curve (AUC) technique for quantifying antioxidant capacity, thus combining both the inhibition time and inhibition degree of the free radical action by antioxidants into a single quantity (20–22). The ORAC assay has been used by different laboratories (23–28) and has provided significant information about the antioxidant capacity of various biological samples

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[2] Mention of a trade name, proprietary product, or specific equipment does not constitute a guarantee by the US Department of Agriculture and does not imply its approval to the exclusion of other products that may be suitable.

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Received October 7, 1997.

Accepted for publication May 7, 1998.

PM3006722648

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from pure compounds, such as melatonin (23), dopamine (25), and flavonoids (26, 29–31), to complex matrixes, such as tea (19, 26), fruit (32), vegetables (19), herbs (27), and animal tissues (24, 28, 33–35).

SUBJECTS AND METHODS

Subjects

Eighteen young subjects (aged 20–40 y; 9 men and 9 women) and 18 old subjects (aged 60–80 y; 9 men and 9 women) were recruited from the New England region. All study participants were in good health as determined by a medical history questionnaire, physical examination, and results of clinical laboratory tests. All of the subjects fulfilled the following eligibility criteria: *1*) they had no history of cardiovascular, hepatic, gastrointestinal, or renal disease; *2*) they had not been alcoholic; *3*) they had not used antibiotics or supplemental vitamin or minerals within ≥4 wk before the start of the study; and *4*) they did not smoke. Women were specifically excluded from the study if they were pregnant, were lactating, were using exogenous hormones, or had a history of menstrual irregularities. The study protocol was approved by the Human Investigation Review Committee of Tufts University and the New England Medical Center and written, informed consent was obtained from each study participant. This study was designed to examine the plasma phylloquinone and carotenoid responses to diet as reported elsewhere (36, 37). The current report focuses on the effects of a diet rich in fruit and vegetables on plasma antioxidant capacity.

Controlled diets

Each study participant resided in the Metabolic Research Unit (MRU) at the Jean Mayer US Department of Agriculture Human Nutrition Research Center on Aging at Tufts University for two 15-d periods. The order of dietary manipulation was determined by a crossover design. There was a free-living period of a minimum of 6 wk between the 2 residency periods, when each subject consumed a self-selected diet.

The diet consumed throughout the residency periods was a 3-d rotating plan based on foods that are commonly consumed by Americans (38). Each subject consumed diet A in one period and diet B in another period. Diet A provided an average of 10 servings of fruit and vegetables each day for 15 d, as shown in **Table 1**. The daily intake of total antioxidants from the fruit and vegetables was 3.30 mmol Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid, a water-soluble vitamin E analogue; Aldrich, Milwaukee) equivalent, as determined by using the ORAC assay (Table 1). Diet B was the same as diet A, except diet B also provided a 102.4-g serving of microwave-heated broccoli at both lunch and dinner (which provided an ORAC value of 0.78 mmol Trolox equivalent) on days 6–10. Thus, diet B provided 12 servings of fruit and vegetables each day on days 6–10. All meals were prepared under the supervision of a dietitian at the MRU. With the exception of water, no other food or beverages were allowed during the residency period. When averaged over 3 d for diet A, the percentages of energy from protein, fat, and carbohydrate were 16%, 26%, and 58%, respectively. All study participants maintained their usual physical activities. They also maintained their body weights, which were measured daily during both study periods.

TABLE 1
Intake [total and oxygen radical absorbance capacity (ORAC)] from fruit and vegetables in the 3-d rotating diet (diet A)

Day and fruit and vegetables provided	Amount	ORAC[1]
	g	*μmol*
Day 1		
Vegetable juice[2]	250	745
Apple juice[3]	124	309
Peach juice[4]	150	375
Lemon juice[2]	15	38
Jelly, grape[2]	15	27
Corn[3]	100	400
Pears[3]	100	130
Carrots[3]	100	210
Pumpkin muffin[2]	100	320
Banana[3]	100	220
Green beans[3]	50	100
Garlic powder[4]	0.6	12
Total		2886
Day 2		
Vegetable juice[2]	250	745
Apple sauce[4]	100	250
Tomato sauce[2]	50	149
Peas[2]	125	450
Orange[3]	150	1125
Green beans[3]	100	200
Cauliflower[3]	100	380
Onion[3]	10	45
Total		3344
Day 3		
Vegetable juice[2]	250	745
Cranberry juice[2]	126	200
Jelly, grape[2]	15	27
Corn[3]	100	400
Pears[3]	100	130
Pumpkin muffin[2]	100	320
Green beans[3]	150	300
Carrots[3]	100	210
Orange[3]	150	1125
Onion[3]	10	45
Garlic powder[3]	0.3	6
Tomato sauce[2]	50	149
Total		3657

[1] Trolox (Aldrich, Milwaukee) equivalent.
[2] Unpublished data for ORAC.
[3] Data for ORAC content from Cao et al (19) and Wang et al (32).
[4] Estimated ORAC content.

Usual dietary intakes during the previous 12 mo were estimated by using a semiquantitative food-frequency questionnaire (39, 40). The total daily number of servings of fruit and vegetables per subject were then tabulated according to standard serving sizes described on the questionnaire.

Plasma antioxidant capacity and individual antioxidant analysis

Fasting blood samples were obtained from subjects before breakfast. Plasma antioxidant capacity was determined by ORAC assay using citrated plasma deproteinized with 0.5 mol $HClO_4$/L (1:1, vol:vol). The automated ORAC assay was carried out on a COBAS FARA II spectrofluorometric analyzer (Roche Diagnostic System Inc, Branchburg, NJ) with fluorescent filters

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(excitation wavelength: 540 nm; emission wavelength: 565 nm) as described previously (21). Briefly, in the final assay mixture (0.4 mL total volume) *R*-phycoerythrin (*R*-PE; 16.7 nmol/L) was used as a target of free radical attack with 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH; 4 mmol/L) as a peroxyl radical generator. Trolox was used as a control standard. The analyzer was programmed to record the fluorescence of *R*-PE every 2 min after AAPH was added. All fluorescence measurements were expressed relative to the initial reading. Final results were calculated by using the differences of areas under the *R*-PE decay curves between the blank and a sample, and expressed as μmol Trolox equivalent/L (21). Plasma α-tocopherol concentrations were determined by HPLC (41) in the Nutrition Evaluation Laboratory of the Jean Mayer US Department of Agriculture–HNRCA at Tufts University.

Statistical analysis

Results are expressed as means ± SEMs. The effects of age, sex, diet (between-subject factors), and time (day; within-subject factor), as well as their interactions were determined by analysis of variance (ANOVA) using SYSTAT (SYSTAT, Inc, Chicago). The significance ($P < 0.05$) of differences in plasma ORAC and α-tocopherol between different days was then determined by multiple paired *t* test with a Bonferroni adjustment. The correlation and regression analyses of plasma ORAC and daily intakes of total antioxidants from fruit and vegetables were computed by using SYSTAT. The regression line was best described by the equation: $Y = a + b\ln X$, as assessed by the correlation coefficient.

RESULTS

Usual daily intake of total antioxidants from fruit and vegetables, the baseline fasting plasma ORAC, and α-tocopherol concentrations

The usual number of fruit and vegetable servings consumed by study participants during the year before the study is shown in **Table 2**. These subjects consumed an estimated average of 5 servings of fruit and vegetables per day. The fruit and vegetables used in this estimation included fruit juices, grapes or raisins, prunes, bananas, cantaloupe, watermelon, apples, pears, oranges, grapefruit, strawberries, blueberries, peaches, apricots, plums, tomatoes, tomato sauce, tofu, string beans, broccoli, cabbage or cole slaw, cauliflower, Brussels sprouts, carrots, corn, peas, lima beans, beans or lentils, yellow squash, eggplant, zucchini or other summer squash, yams or sweet potatoes, spinach, kale, mustard or chard greens, iceberg or head lettuce, celery, beets, alfalfa sprouts, red chili sauce, garlic, and vegetable juices. The average daily intake of total antioxidants from these fruit and vegetables was 1.67 mmol Trolox equivalent (Table 2). No significant effects of age or sex were found.

Baseline fasting plasma ORAC and α-tocopherol concentrations of the study participants before starting diet A or diet B are shown in **Table 3**. No effects of age or sex on the baseline plasma ORAC were found by using two-way ANOVA. However, there was a significant age effect for α-tocopherol ($P < 0.001$); young subjects had significantly lower fasting plasma α-tocopherol concentrations than old subjects, as was reported elsewhere (42). The baseline plasma ORAC or plasma α-tocopherol concentration measured before diet A was not significantly different from that measured before diet B.

There was a mean (±SD) of 3.23 ± 1.41 mo (range: 1.5–6 mo) between the 2 periods of residency. The baseline fasting plasma ORAC varied from one subject to another, but was stable for the same subject over the period of 1.5–6 mo. Quartiles depicting the mean (±SEM) baseline fasting plasma ORAC for the first and second times of residency are shown in **Figure 1**. The mean ORAC for the first (highest) quartile was ≈2 times that for the fourth (lowest) quartile. The mean ORAC for the third quartile was increased from the first time to the second time of residency. However, this was primarily because of 2 subjects whose baseline ORAC values increased by 60% and 41%, respectively.

A significant correlation was found in these subjects between their baseline fasting plasma ORAC and their usual daily intakes of total antioxidants from fruit and vegetables, as estimated for the previous year. Daily intakes of total antioxidants were calculated by using the ORAC values of these fruit and vegetables (Table 2). The regression line was described by the equation: $Y = 125.7 + 65.6 \ln X$; $r = 0.44$ ($P = 0.011$). The fasting plasma ORAC (Y) reached a plateau when daily intake of total antioxidants (X) during the previous year was >3.0 mmol Trolox equivalent.

Response of plasma ORAC to the diets rich in fruit and vegetables

Results of ANOVA using age, sex, and diet as between-subject factors and time (day) as a within-subject factor showed that there was a significant effect of time ($P < 0.001$) as well as a significant interaction between time and age ($P < 0.05$) on the plasma ORAC. The interaction between time and sex or between time and diet was not significant.

Response of plasma ORAC to diet A

Plasma antioxidant capacities of both young and old subjects responded to diet A, which provided 10 servings of fruit and vegetables per day over a 15-d period. The changes in plasma ORAC of these subjects on day 1 (baseline), 6, 11, and 16 with diet A

TABLE 2
Average daily fruit and vegetable servings and total antioxidant intake by study participants during 1 y before the study[1]

	Old men ($n = 8$)	Old women ($n = 8$)	Young men ($n = 9$)	Young women ($n = 9$)	All subjects ($n = 34$)
Fruit	2.42 ± 0.39	2.43 ± 0.43	2.30 ± 0.35	2.57 ± 0.70	2.43 ± 0.24
Vegetables	2.56 ± 0.69	3.00 ± 0.39	2.52 ± 0.58	2.25 ± 0.45	2.57 ± 0.26
Total	4.98 ± 0.91	5.43 ± 0.75	4.82 ± 0.59	4.82 ± 1.06	5.00 ± 0.41
ORAC (mmol Trolox equivalent)[2]	1.91 ± 0.46	1.64 ± 0.25	1.42 ± 0.18	1.74 ± 0.58	1.67 ± 0.20

[1] $\bar{x}$ ± SEM. Usual dietary intakes during the previous 12 mo were estimated by using a semiquantitative food questionnaire (39, 40). Two subjects did not complete the questionnaires but did participate in the study.

[2] Calculated by using our published data (19, 32) and the data in Table 1. The following unpublished oxygen radical absorbance capacity (ORAC) data [μmol Trolox (Aldrich, Milwaukee) equivalent/g] were also used in the calculations: raisins, 28.3; prunes, 57.7; cantaloupe, 2.5; watermelon, 1.0; blueberries, 20.3; peaches, 1.6; apricots, 1.6.

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TABLE 3
Baseline plasma oxygen radical absorbance capacity (ORAC) and α-tocopherol concentrations of study participants before starting either diet A or diet B[1]

	Old men	Old women	Young men	Young women
ORAC (μmol Trolox equivalent)[2]				
Diet A	593.0 ± 20.5	567.6 ± 45.3	638.9 ± 40.0	544.9 ± 42.1
Diet B	632.4 ± 30.6	606.3 ± 47.4	631.7 ± 48.7	546.7 ± 42.8
Average	612.7 ± 24.4	587.0 ± 41.1	635.3 ± 33.3	545.8 ± 41.5
α-Tocopherol (μmol/L)[3]				
Diet A[4]	26.1 ± 1.30	25.1 ± 1.78	19.6 ± 0.96	17.4 ± 1.42
Diet B[4]	24.4 ± 1.30	26.1 ± 1.68	18.6 ± 1.56	17.4 ± 0.90
Average[4]	25.2 ± 0.91	25.6 ± 1.19	19.1 ± 0.85	17.4 ± 0.82

[1] $\bar{x}$ ± SEM. Diet A provided 10 servings of fruit and vegetables per day for 15 d. Diet B was the same as diet A, except diet B also provided 2 servings of microwave-heated broccoli (102.4 g at lunch and 102.4 g at dinner, containing 0.78 mmol Trolox equivalent/d) each day on days 6–10.

[2] n = 9 in each group, except for young men, which had 7 subjects; 2 young men were not included because of the unavailability of baseline plasma samples collected before either diet A or diet B for the ORAC assay.

[3] One old man and one old woman were not included because of their high, outlying plasma α-tocopherol concentrations.

[4] Significant effect of age, $P < 0.001$ [two-way (age and sex) ANOVA].

are shown in **Figure 2**. The plasma ORAC values of young subjects on days 6 and 11 were significantly higher than their baseline values. The plasma ORAC values of old subjects on days 11 and 16 were significantly higher than their baseline values.

Response of plasma ORAC to diet B

The changes in plasma ORAC of the subjects on days 1 (baseline), 6, 11, and 16 with diet B are also shown in Figure 2. A significant increase in plasma antioxidant capacity was observed on days 11 and 16 in old subjects consuming the diet, which provided 10 servings of fruit and vegetables per day from days 1 to 15 (same as diet A) and 2 additional servings of broccoli per day from days 6 to 10. The plasma ORAC of young subjects consuming diet B tended to increase, but the changes were not significant. The consumption of additional broccoli from days 6 to 10 had no further effect on the plasma antioxidant capacity. The

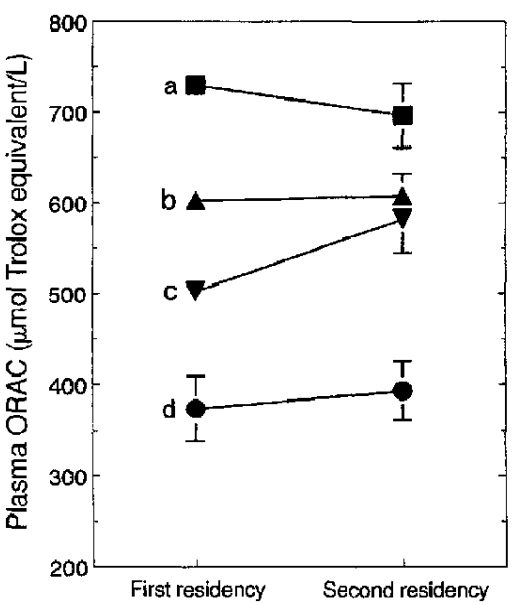

FIGURE 1. Quartiles depicting baseline fasting plasma oxygen radical absorbance capacity (ORAC). The error bars for a, b, and c for the first time of residency are too small to be seen. The quartiles were based on the first time of residency. There was an average of 3.23 ± 1.41 mo (range: 1.5–6 mo) between the first and the second time of residency. a: The first (highest) quartile, n = 8; b: the second quartile, n = 13; c: the third quartile, n = 10; d: the fourth (lowest) quartile, n = 3. Two young men were not included because of the unavailability of baseline plasma samples in either the first time or the second time of residency for the ORAC assay. $\bar{x}$ ± SEM.

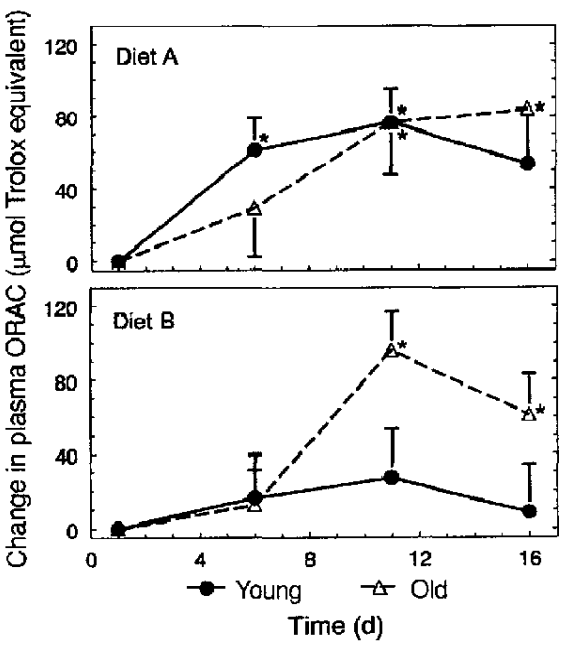

FIGURE 2. Changes in plasma oxygen radical absorbance capacity (ORAC) after consumption of diets high in fruit and vegetables. Diet A provided 10 servings of fruit and vegetables per day for 15 d. Diet B was the same as diet A, except diet B also provided 2 servings of microwave-heated broccoli (102.4 g at lunch and 102.4 g at dinner, containing 0.78 mmol Trolox equivalent/d) each day on days 6–10. *Significantly different from baseline (day 1), $P < 0.05$ (Bonferroni adjusted). The baseline (day 1) plasma ORAC (μmol Trolox equivalent/L) for diet A: young (n = 16), 573.8 ± 30.2; old (n = 18), 585.9 ± 23.9; and for diet B: young (n = 16), 621.4 ± 33.7; old (n = 18), 613.8 ± 28.3. $\bar{x}$ ± SEM.

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change in plasma ORAC from days 6 to 11 with diet B was not significantly different from that with diet A in these young or old subjects.

Response of plasma α-tocopherol to the diets rich in fruit and vegetables

The metabolic diets used in this study were found to contain 23.7 mg total tocopherols/d (mainly in the tocopherol-fortified breakfast cereal), which was 2.4 times the mean dietary intake of α-tocopherol equivalents (9.7 mg/d) in these subjects before entering the study (28). Therefore, the plasma α-tocopherol concentration was also determined in these subjects.

Results of ANOVA using age, sex, and diet as between-subject factors and time (day) as a within-subject factor showed that there was a significant effect of sex, age, and time on the plasma α-tocopherol concentration. The male subjects had higher plasma α-tocopherol concentrations than the female subjects ($P < 0.01$), and the old subjects had higher plasma α-tocopherol concentrations than the young subjects ($P < 0.01$). The effects of diet and the interactions between diet, time, and age on plasma α-tocopherol were not significant.

The changes in plasma α-tocopherol of these subjects on days 1 (baseline), 6, 11, and 16 during diets A and B are shown in **Figure 3**. A significant increase in plasma α-tocopherol concentration was seen in young subjects on days 6, 11, and 16 with both diet A and diet B. The increase in plasma α-tocopherol concentration in old subjects was significant only on day 11 during diet B. However, when young and old subjects were combined, the plasma α-tocopherol concentrations of these subjects on days 6, 11, and 16 were significantly higher than their baseline concentrations during either diet A or diet B (ANOVA: effect of time, $P < 0.01$; effect of diet, $P = 0.267$; time × age, $P = 0.559$).

A significant linear correlation between the change in plasma ORAC value and the change in plasma α-tocopherol concentration was found for young, but not old subjects consuming diet A. No correlation was found for diet B.

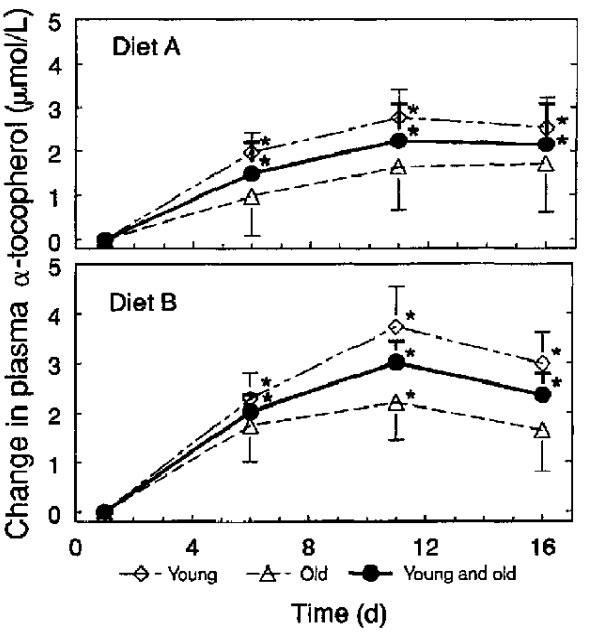

FIGURE 3. Changes in plasma α-tocopherol concentration after consumption of diets high in fruit and vegetables. Diet A provided 10 servings of fruit and vegetables per day for 15 d. Diet B was the same as diet A, except diet B also provided 2 servings of microwave-heated broccoli (102.4 g at lunch and 102.4 g at dinner, containing 0.78 mmol Trolox equivalent/d) each day on days 6–10. *Significantly different from baseline (day 1), $P < 0.05$ (Bonferroni adjusted). The baseline (day 1) plasma α-tocopherol concentration (μmol/L) for diet A: young (n = 18), 18.5 ± 0.86; old (n = 16), 25.6 ± 1.13; and for diet B: young (n = 18), 18.0 ± 0.85; old (n = 16), 25.2 ± 1.11. $\bar{x}$ ± SEM.

DISCUSSION

Almost without exception, epidemiologic studies have found fruit and vegetables to be protective against cancer as well as a range of other diseases (43). *The Dietary Guidelines for Americans* (44), published jointly by the US Department of Agriculture and Department of Health and Human Services, not only advocates fruit and vegetable consumption explicitly, but also specifies that the daily diet should include 2–3 servings of fruit and 3–5 servings of vegetables. However, it was reported that very few individuals in the United States even approached the recommended intakes (43).

The results of the present study provide further evidence to encourage Americans to eat more fruit and vegetables. Results from this study showed that daily intake of the total antioxidants from fruit and vegetables in human subjects was significantly correlated with the fasting plasma ORAC, and that increasing consumption of fruit and vegetables from the usual 5 to the experimental 10 servings/d resulted in a significant increase in plasma ORAC. Aerobic life is characterized by a steady formation of prooxidants balanced by a similar rate of their consumption by antioxidants. To maintain this balance, there is a requirement for the continuous supply of antioxidants, and if this is not met, oxidative damage accumulates, resulting in pathophysiologic events. Therefore, it is important to get various antioxidants by eating fruit and vegetables to ensure an efficient antioxidant defense system.

In this study, the plasma ORAC was determined by using AAPH, a peroxyl radical generator. Peroxyl radicals are the most important oxidants physiologically and pathologically. With AAPH as a peroxyl radical generator, the ORAC assay measures all known nonenzymatic antioxidants, whether they are water- or lipid-soluble. Examples of these antioxidants include ascorbic acid, α-tocopherol, β-carotene, glutathione, methionine, uric acid, bilirubin, phenolic acids, flavanols, flavonols, flavones, isoflavones, flavanones, and anthocyanins (21–23, 26, 29–31).

The increase in plasma antioxidant capacity measured as ORAC after the consumption of high-fruit and -vegetable diets could not be explained solely by the increase in plasma α-tocopherol, which was also observed in the present study. A significant linear correlation between the change in plasma ORAC and the change in plasma α-tocopherol concentration was found for young but not for old subjects consuming diet A. No correlation was found for either young or old subjects consuming diet B. In addition, the significant increases in plasma α-tocopherol concentrations were 1.7–3.7 μmol/L, which accounts for only a small percentage of the significant increases in plasma ORAC, which were 54–95 μmol Trolox equivalent/L (the ORAC value of α-tocopherol is 1 Trolox equivalent). The observed increases

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in plasma α-tocopherol concentrations in the present study were attributed to the tocopherol-fortified breakfast cereal in the diet.

The increase in plasma ORAC also could not be explained by the increases in plasma carotenoids, although the concentrations of plasma lutein, zeaxanthin, α-carotene, β-carotenes (*13-cis*- and *all-trans*-β-carotene), and lycopenes (*9-cis*-, 13-*cis*-, 15-*cis*-, and *all-trans*-lycopene) were significantly increased by ≤457% in these subjects after the consumption of the high-fruit and -vegetable diets (37). The highest plasma concentration of lutein, zeaxanthin, α-carotene, β-carotenes, and lycopenes measured in these subjects over the two 15-d periods of high-fruit and -vegetable consumption were ≈0.55, 0.055, 0.75, 2.25, and 1.2 μmol/L, respectively (37). The ORAC value of β-carotene, which had the highest plasma concentration of the carotenoids in this study group, was even lower than that of α-tocopherol (22).

Plasma vitamin C was not determined in this study. The amount of vitamin C in diet A was 232 mg/d, which was 1.62 times the mean dietary intake of vitamin C (143 mg/d) in these subjects during the 4 d before entering the study. The fasting plasma concentration of this water-soluble antioxidant was not expected to increase substantially after consumption of the high-fruit and -vegetable diet. The fasting plasma concentration of vitamin C in healthy human subjects is ≈30–40 μmol/L (45, 46). Supplementation with vitamin C at 500 mg/d for 2 wk and 2000 mg/d for an additional 2 wk in nonsmoking men and women (18–50 y of age) resulted in only a 55% increase in the fasting plasma vitamin C concentration (46). Therefore, vitamin C may contribute to the increased plasma ORAC observed in this study, but should not be the main contributor (the ORAC value of sodium ascorbate is 0.52–1.0 Trolox equivalent) (20, 22). Fruit and vegetables contain various antioxidants, including vitamin C and β-carotene. However, we have found that in general, >80% of the antioxidant capacity measured in fruit and vegetables (19, 32) is associated with ingredients other than vitamin C, indicating the presence of other potentially important antioxidants in these fruit and vegetables.

Flavonoids and other phenolic compounds appear to be some of the other antioxidants that contribute to the high antioxidant capacity measured in some fruit and vegetables (31); there are >4000 different flavonoids present in plants, and many of them have antioxidant activities that are several times stronger than those of vitamins E and C (29, 30, 47, 48). These phenolic compounds have already been implicated in the protection by fruit and vegetable consumption against diseases (49–52).

Diets A and B used in the present study were basically the same except diet B also contained 2 servings of broccoli each day on days 6–10. The plasma antioxidant capacity of both young and old subjects responded to diet A. However, only old subjects showed a response to diet B in their plasma antioxidant capacity. The plasma ORAC of young subjects showed a nonsignificant trend toward increase with diet B. We do not have an explanation for the discrepancy in results between diet A and diet B for the young subjects. However, broccoli consumption is not likely to explain this difference. The results from the regression analysis between baseline fasting plasma ORAC and daily intakes of total antioxidants, as estimated for the previous year, revealed that fasting plasma ORAC reached a plateau when daily intakes of total antioxidants were >3.0 mmol Trolox equivalent. Diet A provided 3.3 mmol Trolox equivalent/d for 15 d in this study. The effect of the diet rich in fruit and vegetables on the fasting plasma ORAC also seems to have reached a plateau. Therefore, it was not surprising that the consumption of 2 additional servings of broccoli from days 6 to 10 had no further effect on the plasma ORAC in these subjects.

It appears that the study participants maintained their lifestyles and did not change their self-selected diets significantly during the free-living period (1.5–6 mo) between the first and second times of residency, because the fasting baseline plasma ORAC of these subjects was stable over the free-living period. The lowest and highest fasting baseline plasma ORAC seen in some subjects at the first time of residency was observed again in the same subjects at the second time of residency.

In conclusion, we found that the fasting baseline plasma ORAC of these subjects was significantly correlated with their daily estimated intake of total antioxidants from fruit and vegetables during the previous year, and that plasma ORAC can be significantly increased by making fruit and vegetables more available in the diet.

Appreciation is expressed to Christine O'Brien, Kristina Nordensten, and Maureen O'Brien-Morse for their technical assistance; to Jacqueline M Charnley for developing the diets; to Robert Russell for his medical assistance in monitoring the volunteers; and to the staff of the Metabolic Research Unit at the HNRC for their assistance in the care of the subjects. We gratefully acknowledge the participation of the volunteers in this study.

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